Comprehensive two-dimensional liquid chromatography and high throughput effect-directed analysis in environmental research

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Chapter 3

Non-target analysis of household dust and laundry dryer lint using comprehensive two-dimensional liquid chromatography coupled with time of flight mass spectrometry

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Abstract

Household dust and laundry dryer lint are important indoor environmental matrices that may have notable health effects on humans due to chronic exposure. However, due to the sample complexity the studies conducted on these sample matrices until now were almost exclusively on the basis of target analysis. In this study, comprehensive two-dimensional liquid chromatography coupled with time-of-flight mass spectrometry (LC × LC-ToF MS) was applied, to enable non-target analysis of household dust as well as laundry dryer lint for the first time. The higher peak capacity and good orthogonality of LC × LC, together with reduced ion suppression in the MS enabled rapid identification of environmental contaminants in these complex sample matrices. A number of environmental contaminants were tentatively identified based on their accurate masses and isotopic patterns, including plasticizers, flame retardants, pesticides, drug metabolites, etc. The identity of seven compounds: tris(2-butoxyethyl) phosphate, tris(2-chloropropyl) phosphate, n-benzyl butyl phthalate, dibutyl phthalate, tributyl phosphate, triethyl phosphate and N, N-diethyl-meta-toluamide was confirmed using two-dimensional retention alignment and their concentrations in the samples were semi-quantitatively determined.

1. Introduction

The indoor environment has increasingly gained attention as an important source of human exposure to environmental contaminants, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), organophosphate flame retardants (OPFRs), poly- and perfluoroalkyl substances (PFASs), plasticizers and pesticides.[1–5] The exposure in the indoor environment can be as much as 1000-fold higher compared to the outdoor environment, due to relatively longer residence time, poorer ventilation and slower degradation of contaminants.[6] The pollution in the indoor environment is believed to be able to contribute to respiratory diseases, cancer, neuropsychological disorders, etc.[7–9] The potential adverse health effects are expected to be more severe for infants and children due to their frequent hand to mouth contact, resulting in higher intake of dust which can contain high amounts of contaminants, indicating

a higher vulnerability.

Dust is one of the most frequently studied matrices in the indoor environment. [10–13] It is a mixture of fibers, dead skin cells, bugs, soil particles, residues of furniture, electronics and other domestic consumer products. Due to the complexity of the matrix, studies on indoor dust are almost exclusively limited to targeted analysis of a specific group of compounds, and based on gas chromatography coupled to mass spectrometry (GC-MS) or liquid chromatography coupled to mass spectrometry (LC-MS).[11,12,14,15] A method for non-target screening of environmental contaminants in indoor dust using comprehensive two-dimensional gas chromatography (GC × GC) coupled to time-of-flight mass spectrometry (ToF-MS) was described by Hilton et al.[16] Using such a comprehensive approach, not only the compounds reported to be present in the dust sample by the National Institute of Standards and Technology (NIST) were detected, but also the presence of several other compounds, e.g. biphenyls and sulfur, nitrogen, and oxygen analogs of PAHs, was observed.

Comprehensive two-dimensional liquid chromatography coupled to time-of-flight mass spectrometry (LC × LC-ToF MS) is an emerging analytical technique that has shown its strength in resolving complex environmental matrices such as wastewater treatment plant (WWTP) effluents.[17–19] The major advantage of LC × LC in environmental analysis compared to one-dimensional LC is the greater peak capacity, multi-selectivity and, therefore, the reduced matrix effect resulting in ion suppression in the MS interface. Furthermore, high resolution MS and two-dimensional retention time alignment strongly support the identification of unknown environmental contaminants. Therefore, LC × LC-ToF MS is a very useful tool for non-target screening of complex environmental samples.

As an alternative matrix for house dust, laundry dryer lint may be used as a proxy for human exposure. The dryer lint contains synthetic fibers, cotton, human hairs and probably residues of laundry detergent. Several studies have been conducted to investigate lead, polybrominated diphenyl ethers (PBDEs), bisphenol A and dioxins in laundry dryer lint.[20–23] However, similar to indoor dust, non-target analysis of this matrix is expected to significantly add to the knowledge on the occurrence of (toxic) chemicals in laundry dryer lint.

In this study, LC × LC-ToF MS was applied to perform non-target screening of environmental contaminants in indoor dust and dryer lint. In order to achieve a better

extraction efficiency of emerging environmental contaminants, a new extraction method based on a previously reported study of PFASs analysis in dust was implemented.[24] Selection of the most interesting samples for LC × LC-ToF MS was done using an acetylcholinesterase (AChE) inhibition assay.

2. Materials and methods

2.1 Chemicals and materials

Perfluorooctanesulfonic acid (PFOS), perfluorononanoic acid (PFNA), perfluorohexane sulfonate (PFHxS), tris(1,3-chloroisopropyl) phosphate (TDCiPP), tris(2-chloropropyl) phosphate (TCPP), N, N-diethyl-meta-toluamide (DEET), dithiobisnitrobenzoic acid (DTNB, Ellman's reagent) and acetylthiocholine iodide (ATC) were purchased from Fluka (Zwijndrecht, the Netherlands). Standards of tris(2-chloroethyl) phosphate (TCEP), tris(2-butoxyethyl) phosphate (TBOEP), tributyl phosphate (TBP), triethyl phosphate (TEP), dicyclohexyl phthalate (DCHP), pirimicarb, isoprocarb, propoxur, varenicline and purified acetylcholinesterase (AChE) from electric eel *(Electrophorus electricus)* were obtained from Sigma-Aldrich (Zwijndrecht, the Netherlands). Triisobutyl phosphate (TiBP) was purchased from Merck (Darmstadt, Germany). Trimethacarb and 3,5-xylyl methylcarbamate (XMC) were purchased from LGC standards (Teddington, UK). The standards of n-benzyl butyl phthalate (BBzP) and dibutyl phthalate (DBP) were purchased from Riedel de Haën (Seelze, Germany). The EPA SRM 2585 house dust was from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). The EPA 531.1 carbamate pesticide calibration mixture was purchased from Restek (Bellefonte, PA, USA). Acetonitrile was HPLC grade supplied by Sigma-Aldrich. HPLC water was obtained from a Milli-Q Reference A+ purification system (Millipore, Bedford, MA, USA). Formic acid added to the eluent was ordered from Fluka.

2.2 Instrumentation

The comprehensive LC x LC system deployed an Agilent 1100 HPLC binary pump as the first dimension, an Agilent 1290 Infinity UHPLC binary pump as the second dimension together with an Agilent 1100 auto sampler and an Agilent 1290 Infinity thermostatted column compartment (TCC) with a 2-position/4-port duo valve and

two sampling loops (80 µL) installed as the 2D interface (Agilent Technologies, Waldbronn, Germany). The LC × LC module was controlled and monitored by Chemstation version B.04.03 (Agilent Technologies) with 2D-LC add-on. A ZORBAX Eclipse Plus (1.8 µm, 2.1 × 150 mm ID) C18 Rapid Resolution HD column (Agilent Technologies, Santa Clara, CA, USA) was used in the first dimension and a Kinetex pentafluorophenyl (PFP) column (2.6 µm, 50 × 4.6 mm ID, Phenomenex, Torrance, CA, USA) in the second dimension. After the second column, in order to adapt an optimal flow for the coupled ToF-MS (Bruker micrOTOF, Bremen, Germany), resolving power ~10,000) with an electrospray (ESI) interface, a QuickSplit adjustable flow splitter (Richmond, CA, USA) was applied which directed 20% of the flow to the MS detector. The remaining 80% of the flow was sent to the waste.

2.3 Sample collection

The five dust samples were collected using a Dustream™ dust collector (Indoor Biotechnologies Ltd., Wiltshire, United Kingdom) containing a disposable filter (mesh size 40 µm) and attached on a household vacuum cleaner tube, from five different families in the region of Uppsala, Sweden. The dust collected was so-called still standing dust, from surfaces little influenced by daily life, e.g. no walking and not containing e.g. bread crumbs and soil. The six dryer lint samples were collected from two households in the Netherlands. The lint was taken gently from the filter sheets of the laundry dryers and then wrapped in aluminum foil before extraction and cleanup.

2.4 Sample extraction and cleanup

To better fit the LC × LC-ToF MS based method, the extraction and cleanup procedure was carried out using polar solvents methanol and acetonitrile modified from Fraser et al.[24] Briefly, 50 mg of sample (SRM 2585, house dust or dryer lint) was weighed in a 15 ml disposable polypropylene tube and 5 ml of methanol was added. The tube was vortexed for one minute, followed by ultra-sonication for 15 minutes and centrifugation for 5 minutes at 1500 rpm. The supernatant was transferred to a new polypropylene tube and the same extraction steps were performed on the residue using acetonitrile. The supernatants were combined and filtered over an Envicarb SPE cartridge (Supelco, Zwijndrecht, Netherlands)

activated with two portions of 5 ml methanol and acetonitrile (1:1 v/v). The SPE cartridges were rinsed with 0.5 ml methanol and acetonitrile (1:1 v/v) for four times after sample loading. The extraction and cleanup procedure was validated by spiking 50 µl of PFOS (3 µg/ml), PFNA (3 µg/ml), PFHxS (3 µg/ml), TCEP (3 µg/ml), TDCiPP (3 µg/ml) and TBOEP (30 µg/ml) into 50 mg SRM 2585 house dust. The recovery of each compound was determined (n=3) 100% (±21%), 70% (±18%), 85% (±18%), 62% (±5%), 47% (±2%) and 65% (±19%) respectively. Prior to the LC × LC-ToF MS analysis, the extract was reduced to a volume of 0.2 ml under a gentle nitrogen flow and 0.4 ml of Milli-Q water was added.

2.5 Bioassays screening for sample prioritization

The biological activity of the sample extracts (n=11) was analysed using the acetylcholinesterase (AChE) inhibition assay, to select the most interesting samples for LC × LC-ToF MS based non-targeted analysis. The assay was applied as described earlier.[19] The AChE inhibition observed in the household dust samples (n=5) was 0-24% and in the laundry dryer lint samples (n=6) an inhibition of 28-86% was measured. Finally, one household dust sample and one dryer lint sample with highest level of inhibition were selected for LC × LC-ToF MS analysis.

2.6 Chromatographic and mass spectrometric conditions

The LC × LC conditions are given in Table 1. In the second dimension, continuous shift gradient (the gradient program continuously changed in each modulation period according to the concurrent mobile phase combination of the elute from the first dimension) was used to achieve the optimal separation. The start and stop signals of the micrOTOF were initiated by the LC × LC system through external control via serial ports. The MS data were recorded using a scan frequency of 5 Hz by Bruker OtofControl 3.0 (Bruker Daltonics), to ensure enough data points for fast separation in the second dimension. The ion source and transfer settings of the MS were optimized in the mass range m/z 50−1000. The capillary voltage of the ESI was 4500 V with end plate offset −500 V. The nebulizer gas (N_2) was operated at 4.0 bar and the drying gas was set at 8 L/min at a temperature of 200 °C. The capillary exit was operated at 100 V with a skimmer voltage of 33.3 V, the hexapole RF was regulated to 90 Vpp and lens 1 prepulse storage was set to 1 µs.

Table 1. LC × LC conditions.

Column combination	Injection volume	First dimension LC conditions	Second dimension LC conditions
C18 × PFP	20 µl	Mobile phase: (A) water; (B) acetonitrile. Gradient: 0 min 30% B, 50 min 90% B, 60 min 90% B. Flow rate: 0.1 ml/min.	Mobile phase: (A) water with 0.1% formic acid; (B) acetonitrile with 0.1% formic acid. Modulation time: 0.6 min. Gradient: 0 min 30% B, shifted to 85% in 55 min and then 80% at 55 min; 0.5 min 50% B, shifted to 95% in 40 min; 06 min 50% B, shifted to 95% in 40 min. Flow rate: 2.0 ml/min.

2.7 Data analysis

The MS data were calibrated using the high precision calibration (HPC) method from the instrument software package DataAnalysis (version 4.1, Bruker Daltonics) on a calibration tunemix solution introduced prior to each analysis. The calibrated MS data were saved in netCDF format and visualized as contour plots using the linear interpolation algorithm in the GC Image software (version 2.3b4, Lincoln, NE, USA). Following this, the blob (2D peak) detection function in the GC Image software was applied. The threshold was set at 10,000 for the dust sample and at 4,000 for the laundry dryer lint sample. The retention times of both dimensions were normalized and assessed for orthogonality according to previous work.[17] Non-target analysis was performed using the SmartFormula function in DataAnalysis (Bruker software), to determine the possible chemical formulas of the MS peaks based on their detected accurate masses and mSigma value (The mSigma value is the goodness of fit between measured and theoretical isotopic pattern, A lower mSigma value indicates a better fit). The proposed formulas were first checked in online databases of ChEBI (Chemical Entities of Biological Interest), KEGG (Kyoto Encyclopedia of Genes and Genomes) and METLIN, using the CompoundCrawler function in DataAnalysis. As we focused on compounds which are biological active and therefore may impact the ecosystem or human health. If no match was found, the formula was checked in Chemspider and the matched compounds which had trade names were taken as candidates, which means they are synthetic compounds (e.g. pharmaceuticals, pesticides, flame retardants and plasticizers) which possibly exist in our environment. The semi-quantification was performed by fitting the volume of

the 2D chromatographic peaks of the confirmed compounds in the sample runs to their corresponding one-point standard calibration curves. Their estimated concentrations in the original samples were calculated based on the amount of sample used, the recovery of the extraction, the injection volume and split setting.

3. Result and discussion

3.1 Chromatographic separation

A stationary phase combination of C18 and PFP was selected for the two separation dimensions due to good orthogonality as well as solvent compatibility.[17,19] A fast gradient in the second dimension was optimized. For both samples, good separation in the second dimension was observed (Figures 1A and 1B), indicating an enhancement of the peak capacity compared with traditional 1D-LC. The orthogonality of the separation was estimated for both samples using a previously reported surface coverage algorithm.[17] For the household dust chromatogram, the calculated orthogonality was 67% and for the dryer lint chromatogram 73%, based on the surface coverage in Figure 1C and 1D, respectively. The orthogonality is lower than the recent study of WWTP effluent sample,[19] possibly due to the limitation of ionization capability of hydrophobic chemicals, which are expected to be present in higher concentrations in the dust and dryer lint samples.

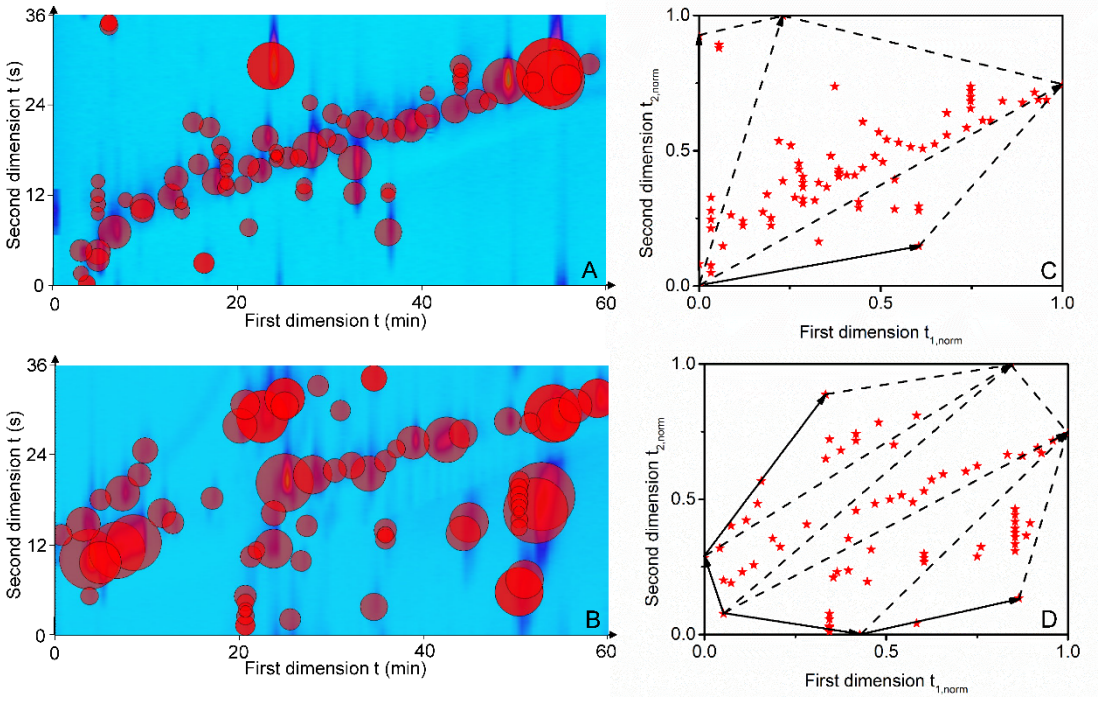

Figure 1. Contour plot of LC × LC-ESI (+)-ToF MS chromatograms of the house dust sample (A) and the dryer lint sample (B) and their orthogonality estimation using the surface coverage method (C, D). The blob (red circular) detection was performed according to section 2.7.

3.2 Identification of compounds in house dust

The good separation of the compounds in the LC × LC system led to an easier identification because of the reduced matrix effect. The tentatively identified compounds in the dust sample using non-target identification are listed in Table 2. In total 16 compounds could be identified, representing a wide range of compound groups with diverse functionalities. OPFRs and phthalates are known environmental contaminants present in house dust. OPFRs in house dust have been associated with alterations in hormone levels and decreased sperm counts.[25] Phthalates are also endocrine disruptors which can inhibit testosterone synthesis [26] and their levels in house dust have been associated with asthma and allergic symptoms in children.[9] Other tentatively identified compounds are rarely reported in house dust. DEET (N, N-diethyl-meta-toluamide), as the world's most widely used insect repellent, is a major effective ingredient in e.g. anti-mosquito spray. The compound is highly skin-permeable and the exposure was reported to be associated with encephalopathy, particularly for children.[27] Azelaic acid and sebacic acid are widely applied as plasticizers, as monomers of polyamide, in personal care products such as skin cream and hair conditioner.[28] Interestingly, some biocides (pirimicarb, metolcarb, and benomyl) were identified, which may have been used indoors or came from indoor plants or flowers.

Table 2. Non-target screening results of the household dust sample.

Suggested Compound	CAS	Mol. Formula	Ion Form	Err. [ppm]	mSigma	m/z Calc.	m/z Meas.	Possible Source
Phenylalanine*	150-30-1	$C_9H_{12}NO_2$	$[M+H]^+$	-4.5	3.5	166.0863	166.0870	Natural product
Metolcarb*	1129-41-5	$C_9H_{12}NO_2$	$[M+H]^+$	-4.5	3.5	166.0863	166.0870	Insecticide
Streptidine	85-17-6	$C_8H_{19}N_6O_4$	$[M+H]^+$	7.8	8.7	263.1462	263.1483	Metabolite of streptomycin
Azelaic acid	123-99-9	$C_9H_{17}O_4$	$[M+H]^+$	-6.8	13.9	189.1121	189.1134	Drug, plasticizer and lubricant
Sebacic acid	111-20-6	$C_{10}H_{19}O_4$	$[M+H]^+$	6.8	9.7	203.1278	203.1292	Foam seating and bedding products
Triisobutyl phosphate (TiBP)*	126-71-6	$C_{12}H_{28}O_4P$	$[M+H]^+$	2.0	26.6	267.1720	267.1725	Flame retardant
Tributyl phosphate (TBP)*	126-73-8	$C_{12}H_{28}O_4P$	$[M+H]^+$	2.0	26.6	267.1720	267.1725	Flame retardant, plasticizer
Tris (2-butoxyethyl) phosphate (TBOEP)	78-51-3	$C_{18}H_{40}O_7P$	$[M+H]^+$	10.7	10.6	399.2506	399.2549	Flame retardant
Tris(1-chloro-2-propanyl) phosphate (TCPP)	13674-84-5	$C_9H_{19}Cl_3O_4P$	$[M+H]^+$	5.2	29.4	327.0081	327.0098	Flame retardant
Dibutyl phthalate (DBP)*	84-74-2	$C_{16}H_{23}O_4$	$[M+H]^+$	2.0	19.0	279.1591	279.1596	Plasticizer
Diisobutyl phthalate (DiBP)*	84-69-5	$C_{16}H_{23}O_4$	$[M+H]^+$	2.0	19.0	279.1591	279.1596	Industrial solvent
Dicyclohexyl phthalate (DCHP)	84-61-7	$C_{20}H_{27}O_4$	$[M+H]^+$	-4.1	33.3	331.1904	331.1918	Adhesive chemical and coating additive
n-Butyl benzyl phthalate (BBzP)	85-68-7	$C_{19}H_{21}O_4$	$[M+H]^+$	-7.9	7.5	313.1434	313.1459	Plasticizer, adhesive and floor covering
Pirimicarb	23103-98-2	$C_{11}H_{19}N_4O_2$	$[M+H]^+$	5.6	31.8	313.1503	239.1489	Insecticide
N,N-Diethyl-meta-toluamide (DEET)	134-62-3	$C_{12}H_{18}NO$	$[M+H]^+$	6.3	21.4	192.1383	192.1395	Active ingredient in insect repellents
Benomyl	17804-35-2	$C_{14}H_{19}N_4O_3$	$[M+H]^+$	-19.5	28.8	291.1452	291.1395	Fungicide

Acronyms: Mol.-molecular; Err.-mass error; Calc.-calculated; Meas.-measured

*Peaks m/z 267.1725, m/z 166.0870 and m/z 279.1596 had two possible isomers.

3.3 Identification of compounds in dryer lint

A non-target LC × LC-ToF MS analysis was also carried out for a laundry dryer lint sample (Table 3). To our knowledge, laundry dryer lint has not been explored by non-target analysis yet. However, clothes as possible sources for laundry dryer lint may have considerable exposure to the human body. In addition, sucking behavior of infants may cause exposure to chemicals contained in the clothing fabric. Possible sources of chemicals in dryer lint include but are not limited to textile debris, clothes dyes, laundry detergent additives, surfactants and flame retardants. The tentatively identified compounds in the dryer lint sample analyzed cover a broad spectrum of chemicals including pesticides, therapeutic as well as illicit drugs, fragrances, plasticizers and compound metabolites (Table 3).

Interestingly, a large number of pesticides were detected in the dryer lint. The carbamate pesticides isoprocarb, trimethacarb, pirimicarb, propoxur and 3,5-xylyl methylcarbamate (XMC), the anticholinergic medicine oxybutynin and the plant metabolite belladine are acetylcholinesterase inhibitors which may explain the relatively high AChE inhibition detected in the sample. The compound triethyl phosphate (TEP) is a polymer resin modifier, a strength agent for rubber and plastic including vinyl polymers and unsaturated polyesters and a flame retardant.

Table 3. Non-target screening results of the laundry dryer lint sample.

Suggested Compound	CAS	Mol. Formula	Ion Form	Err [ppm]	mSigma	m/z Calc.	m/z Meas.	Source
N,N-Diethyl-meta-toluamide (DEET)	134-62-3	$C_{12}H_{17}NO$	$[M+H]^+$	-3.2	16.8	192.1383	192.1389	Insect repellent
Diphenylamine	122-39-4	$C_{12}H_{11}N$	$[M+H]^+$	4.6	32.1	170.0964	170.0956	Fungicide
Isoprocarb*	2631-40-5	$C_{11}H_{15}NO_2$	$[M+H]^+$	14.7	9.1	194.1176	194.1147	Carbamate insecticide
Butyl anthranilate*	7756-96-9	$C_{11}H_{15}NO_2$	$[M+H]^+$	14.7	9.1	194.1176	194.1147	Fragrance and insect repellent
Trimethacarb*	12407-86-2	$C_{11}H_{15}NO_2$	$[M+H]^+$	14.7	9.1	194.1176	194.1147	Carbamate insecticide
MDMA*	42542-10-9	$C_{11}H_{15}NO_2$	$[M+H]^+$	14.7	9.1	194.1176	194.1147	Drug of abuse
Pirimicarb	23103-98-2	$C_{11}H_{18}N_4O_2$	$[M+H]^+$	-11.2	20	239.1503	239.1476	Carbamate insecticide
Propoxur	114-26-1	$C_{11}H_{15}NO_3$	$[M+H]^+$	-10.4	7.7	210.1125	210.1103	Carbamate insecticide
Butoctamide semisuccinate (JAN)	32838-28-1	$C_{16}H_{30}NO_5$	$[M+H]^+$	0.6	7.7	316.2118	316.2117	Non-barbiturate drug
Spiroxamine	118134-30-8	$C_{18}H_{35}NO_2$	$[M+H]^+$	-6.2	23.8	298.2741	298.2722	Fungicide
Triethyl phosphate	78-40-0	$C_6H_{16}O_4P$	[M+H]+	0,4	7,8	183.0781	183.0780	Plasticizer
3,5-Xylyl methylcarbamate	2655-14-3	$C_{10}H_{13}NO_2$	$[M+H]^+$	17.1	37.9	180.1019	180.0988	Carbamate insecticide
Xestoaminol C	129825-28-1	$C_{14}H_{32}NO$	$[M+H]^+$	2.9	15.5	230.2478	230.2472	Nature product
Belladine	501-06-4	$C_{19}H_{26}NO_3$	$[M+H]^+$	-6.9	26.3	316.1907	316.1929	Plant metabolite
Decanamide	2319-29-1	$C_{10}H_{22}NO$	$[M+H]^+$	2.3	8.6	172.1696	172.1692	Surfactant
Oxybutynin	5633-20-5	$C_{22}H_{32}NO_3$	$[M+H]^+$	-5.9	24.8	358.2377	358.2398	Anticholinergic medicine
Linoleamide	3072-13-7	$C_{18}H_{34}NO$	$[M+H]^+$	3.5	13.1	280.2635	280.2625	Endogenous lipid, human metabolite
o-Palmitoyl-L-carnitine	2364-67-2	$C_{23}H_{46}NO_4$	$[M+H]^+$	-1.8	26	400.3421	400.3429	Human metabolite
4-Decylbenzenesulfonic acid	1322-98-1	$C_{16}H_{27}O_3S$	$[M+H]^+$	3.2	25.7	299.1675	299.1666	Laundry detergent
Varenicline	249296-44-4	$C_{13}H_{14}N_3$	$[M+H]^+$	-7.3	8.8	212.1182	212.1167	Prescription medication used to treat nicotine addiction

Acronyms: Mol.-molecular; Err.-mass error; Calc.-calculated; Meas.-measured

*Peak m/z 194.1147 had four possible isomers.

3.4 Confirmation of tentatively identified compounds in dust and dryer lint

The results shown in Table 2 and Table 3 are tentative because no analytical standards were used to confirm the retention time and mass spectrum. In order to increase the confidence of the identification, a two-dimensional retention time alignment check was performed for a sub-set of the tentatively identified compounds.[17] First, two-dimensional extracted ion chromatograms (EICs) of the tentatively identified peaks were established using the GC Image software. Then standard solutions (5 µg/ml each compound) were analysed with the LC × LC-MS system, applying identical chromatographic conditions as the sample runs. This was followed by comparing the 2D retention times of the standards with the samples (Figure 2). The threshold for accepted deviation was 0.6 min in the first dimension and 2.0 s in the second dimension. This approach showed that m/z=267.172±0.05 was tributyl phosphate (TBP) and not triisobutyl phosphate (TiBP), as the latter had a different first dimension retention time (Figure 2E). Also TBOEP, TCPP, BBzP, DBP, TBP and DEET were confirmed in the house dust sample (Figure 2A, 2B, 2C, 2D and 2F); TEP and DEET were confirmed in the dryer lint sample as well (Figure 2F and 2G). The confirmation experiments were also performed for compounds DCHP, isoprocarb, trimethacarb, pirimicarb, propoxur, XMC and varenicline but the identity of these chemicals could not be confirmed. The confirmation rate of the tentatively identified compounds are 50%.

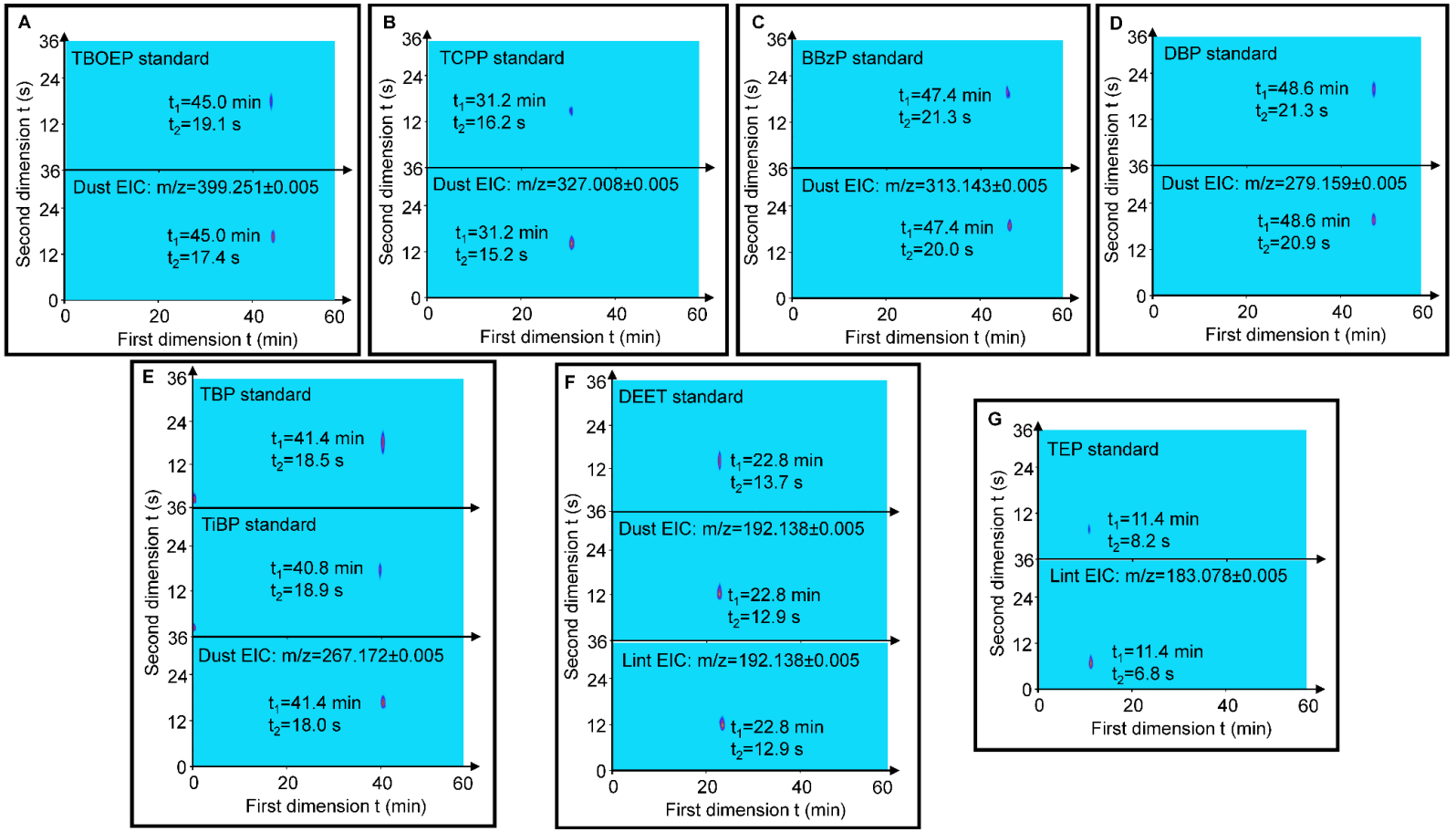

Figure 2. Two-dimensional retention alignment of six compounds identified in the dust sample and two compounds in the lint sample.

The concentrations of the confirmed compounds in the original samples were calculated based on the amount of sample used, the recovery of the extraction, the injection volume and the split setting (Table 4). The semi-quantified results of several OPFRs in the household dust sample corresponded well with the results of target analysis done on the sample using GC-MS (unpublished results). The OPFRs and phthalates have been frequently analysed in household dust sample and their concentrations varied greatly depending on the sampling site.[5,29–31] DEET was previously reported as indoor contaminant but this is the first time it was discovered in laundry dryer lint, with a higher concentration (3-4 fold) than in household dust.[32] The concentration of TEP, found for the first time in dryer lint, was assessed to be 3.6 µg/g. Studies on chicken embryos revealed that TEP is capable of interfering with circulating thyroid hormone levels at concentrations in the order of a few ng/g.[33]

Table 4. Semi-quantitated concentration of the confirmed compounds in the dust and the laundry dryer lint samples.

Confirmed Compound	Concentration (µg/g)
TBP (dust)	0.4
TCPP (dust)	7.6
TBOEP (dust)	6.5
BBzP (dust)	3.9
DBP (dust)	17.0
DEET (dust)	1.2
TEP (dryer lint)	3.6
DEET (dryer lint)	4.1

4. Conclusions

Non-target analysis of house dust and laundry dryer lint in the indoor environment using LC × LC-ToF MS was demonstrated for the first time. Compared to conventional analytical approaches such as single-column GC or one-dimensional

LC, the higher peak capacity and multi-selectivity provided by LC × LC makes it a very suitable method for non-target analysis. Using analytical standards, the application of two-dimensional retention time alignment to confirm the identity of tentatively identified compounds has facilitated the identification of isomers, as was shown for TBP. To achieve the same by regular one-dimensional LC would require a dedicated analytical method for the resolution of isomers, which emphasizes the extraordinary usefulness of LC × LC for the identification of this type of compounds. In addition to the clear advantages of the high chromatographic power of the LC × LC system, ion suppression caused by matrix constituents was significantly reduced due to the improved separation of the peaks from the matrix, which led to straightforward HR-MS identification. Therefore, LC × LC-ToF MS is an effective and efficient tool to explore the chemicals and contaminants present in complex matrices.

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